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Title: Efficacy and safety of intravenous immunoglobulin with rituximab versus rituximab alone in childhood-onset steroid-dependent nephrotic syndrome: protocol for a multicentre randomized controlled trial

Running title: RITUXIVIG trial

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Abstract

Introduction: Guidelines for the treatment of steroid-dependent nephrotic syndrome (SDNS) and frequently-relapsing nephrotic syndrome (FRNS) are lacking. Given the substantial impact of SDNS/FRNS on quality of life, strategies aiming to provide long-term remission while minimizing treatment side-effects are needed. Several studies confirm that rituximab is effective in preventing early relapses in SDNS/FRNS, however the long-term relapse rate remains high (~70% at 2 years). This trial will assess the association of intravenous immunoglobulins (IVIg) to rituximab in patients with SDNS/FRNS and inform clinicians on whether IVIg immunomodulatory properties can alter the course of the disease and reduce the use of immunosuppressive drugs and their side effects.

Methods and Analysis: We conduct an open-label multicentre, randomized, parallel-group in a 1:1 ratio, controlled, superiority trial to assess the safety and efficacy of a single infusion of rituximab followed by IVIg compared to rituximab alone in childhood-onset SDNS. The primary outcome is the occurrence of first relapse within 24 months. Patients are allocated to receive either rituximab alone (375 mg/m²) or rituximab followed by IVIg, which includes an initial immunoglobulin dose of 2g/kg, followed by 1.5g/kg injections once a month for the following five months (Max dose: 100 g).

Ethics and Dissemination: The study has been approved by the Ethics committee (Comité de Protection des Personnes CPP) of Ouest I and authorised by the French drug regulatory agency (Agence Nationale de Sécurité du Médicament et des Produits de Santé, ANSM). Results of the primary study and the secondary aims will be disseminated through peer-reviewed publications.

Trial Registration Number: This trial is registered at ClinicalTrials.gov (NCT03560011)

Key Words: steroid-dependent nephrotic syndrome, immunoglobulin, rituximab

Article Summary

Strengths and Limitations of This Study

- This study will be conducted as a national multicentre randomized controlled trial providing the first reliable data on the use of IVIg in combination with rituximab in patients with idiopathic nephrotic syndrome.
- The lack of blinding of the patients and the physicians is a limitation to the study design, however the objectivity of the primary outcome reduces the risk of bias.
- Intravenous administration of the intervention addresses concerns of non-compliance.

Introduction

Background

Idiopathic nephrotic syndrome (INS) is the first glomerulopathy in children with an incidence estimated between 2 and 3/100,000 inhabitants and a high prevalence of 1/6250 because of the extensive course of the disease. In a recent French cohort including all incident cases in the Paris area, we found a slightly greater incidence of 3.4/100,000 inhabitants under 16 years old.[1] INS is defined by the association of the clinical features of nephrotic syndrome with renal biopsy findings of diffuse foot process effacement on electron microscopy and minimal changes, also called minimal change disease (MCD), or focal segmental glomerulosclerosis (FSGS), or diffuse mesangial proliferation (DMP) on light microscopy.[2] Most patients have histologic findings of MCD. The response to steroid therapy (steroid-sensitive nephrotic syndrome [SSNS] versus steroid-resistant nephrotic syndrome [SRNS]) is of higher prognostic significance than histologic features seen on initial renal biopsy so that renal biopsy is generally not recommended in children with SSNS. Cohort studies including the NEPHROVIR study found that around 90% of the patients are steroid sensitive.[1,3] However, 60% will become steroid-dependent with a major risk of morbidity related to the complications of the relapses (mostly infections due to immunoglobulin loss and thrombosis) and to the side effects of the treatments used in those patients. The pathophysiology of INS is still incompletely understood. In 1974, Shaloub brought evidence for an immune origin of the disease.[4] Since then, standard immunosuppressive drugs such as calcineurin inhibitors or mycophenolate mofetil (MMF) demonstrated the ability to maintain remission while on treatment. Unfortunately, their effect is only suspensive with 75% of relapse after cyclosporine A (CsA) withdrawal [5] and over 90% of relapse after MMF withdrawal,[6] although maintenance of remission is needed to maintain normal renal function in the long run. Cyclophosphamide demonstrated a long-lasting effect in

children with steroid-dependent nephrotic syndrome (SDNS) with a sustained remission rate of 42% at 2 years but its use is limited by its side effects.[7] To date, there is a general agreement on the treatment of the first flare which is based on steroids although differences in dose and length of treatment exist between countries. In France, the national guidelines for the treatment of the first flare was recently changed from 4.5 months of prednisone with a total dose of 3990 mg/m² to 2 months of prednisone with a cumulative dose of 2240 mg/m^2 following the publication of the PREDNOS trial.[8] However, there are currently no guidelines for the treatment of SDNS. Several strategies using low dose steroid therapy (once every other day) and the immunosuppressive drugs mentioned previously have been proposed.[9,10] However, they are associated with significant side effects such as diabetes, high blood pressure, infections and renal fibrosis. Moreover, the long duration of the disease (median time 10 years) has been recently shown to significantly impact the quality of life of patients.[11] Thus treatment and strategies aiming to provide long-term remission while minimizing treatment side-effects in patients with SDNS need to be investigated.

In 2004, rituximab (RTX), a humanized anti-CD20 antibody depleting B cells has been reported to induce sustained remission of the nephrotic syndrome in a patient treated for idiopathic thrombocytopenic purpura.[12] Since then, many reports confirmed that RTX is able to induce long-lasting remission even after B cell recovery in patients with SDNS.[13-15] This finding deeply modified our view on the pathophysiology of the disease with the involvement of B cells and not only T cells as previously described. This implication of B cells is further supported by the strong correlation between B cell recovery and INS relapse in patients relapsing after RTX therapy with a recent report underlying the role of memory B cells (CD19+/CD27+).[16]

Two recent randomized trials demonstrated an improvement of the relapse-free survival with RTX when compared with placebo or long-term steroid therapy.[17,18] Similar results have been found in a recently published French randomized controlled trial NEPHRUTIX since the relapse rates at 6 months was 10% in the rituximab arm compared to 100% in the placebo arm.[19] However, the remission rates after two years in patients treated with RTX is only 30 to 40%. Strategies using repeated RTX injection with long B cell depletion duration greatly increase the relapse-free survival rate to over 60% but increase the risk of infection and persistent hypogammaglobulinemia.[14, 20, 21]

Intravenous immunoglobulin (IVIg), which is used for therapeutic purposes, is a polyspecific immunoglobulin IgG preparation purified from plasma pools of several thousand healthy donors. IVIg preparations primarily contain human IgG molecules, with small amounts of IgA and IgM. The distribution of IgG subclasses in IVIg is comparable to that of IgG in normal serum and the half-life of infused IVIg is approximately three weeks. IVIg was initially used as a substitution for immunoglobulins that were lacking in patients with primary and secondary immune deficiencies. However, since the demonstration in 1981 that IVIg ameliorates immune thrombocytopenic purpura,[22] IVIg is increasingly being used for the treatment of a wide range of autoimmune and systemic inflammatory diseases.[23] In addition to antibody-mediated diseases, IVIg is also effective in several disorders caused by dysregulation of cellular immunity, such as Kawasaki disease, dermatomyositis, multiple sclerosis, graft versus host disease in recipients of allogeneic bone marrow transplants.[24] Clinically, the beneficial effects of IVIg extend beyond the half-life of infused IgG, therefore, its effects cannot be a result of a passive clearance or competition with pathogenic autoantibodies. Together, these observations evoke the possibility that IVIg therapy induces lasting changes in the cellular compartment of the

immune system. Several studies demonstrated the ability of IVIg to modulate B cells immune response in vitro and in vivo through several mechanisms such as apoptosis promotion by modulating BCR signalling after binding to CD22,[25] silencing program induction of B cells and neutralization of cytokines such as the B-cell survival factor (BAFF) and A proliferation inducing ligand (APRIL).[26] In vivo, IVIg therapy in women with recurrent spontaneous abortion is accompanied by a small decrease in the peripheral blood B-cell numbers.[27] Aside from their effects on B cells, IVIg have been found to modulate T cell function especially by expanding and enhancing the functions of regulatory T cells (Treg)[28, 29] and by decreasing T cell activation and proliferation through multiple pathways including Il-2 production inhibition.[30-32] Tha-In et al. found that IVIg were as effective as calcineurin inhibitors to inhibit T cells proliferation in vitro and also impact dendritic cells functions.[33] Many studies also report effects of IVIg on innate immune system.[34]

Thus, we hypothesised that the adjunction of IVIg to a single course of rituximab may further modulate B-cells function and allow a prolonged effect on INS without the need for long-lasting B-cell depletion. Moreover, maintaining a high IgG level may be beneficial in decreasing the risk of infection in those vulnerable patients. Treatment modality was derived from the protocol commonly used to treat antibody-mediated rejection in renal transplant recipients both in adults and in children.[35] In a retrospective pilot study comparing 12 patients treated with RTX and IVIg to 32 controls receiving one injection of RTX alone, we found a great improvement of relapse-free survival at two years from 40% in the RTX alone group to 70% in group receiving both RTX and IVIg with the difference remaining significant after adjustment for age, associated immunosuppressive treatments and B cell depletion duration [unpublished observations, J Hogan]. The proposed clinical trial aims to establish evidence for the use of IVIg

in addition to rituximab in patients with frequently relapsing and steroid-dependant nephrotic syndrome.

Methods/Design

Objectives

Primary objective

Our primary objective is to assess the effect of a single infusion of rituximab followed by immunoglobulin injections (once a month during 5 months) on the occurrence of the first relapse within 24 months following the initiation of treatment in patient with childhood onset SDNS compared to a single infusion of rituximab.

Secondary objectives

To compare the time to first relapse

To compare the total number of relapse over the 24 months of follow-up

To compare the cumulative dose of steroid over the 24 months of follow-up

To compare the tolerance and safety of the two strategies

Our hypothesis is that the adjunction of intravenous immunoglobulin to rituximab to treat patients with SDNS will induce sustained remission of proteinuria even after oral treatment withdrawal and will improve relapse-free survival when compared to rituximab used alone.

Study design (Figure 1)

The trial will be an open-label multicentre, randomized, parallel-group in a 1:1 ratio, controlled, and superiority trial testing a single infusion of rituximab followed by immunoglobulin injections (once a month during 5 months) compared to a single infusion of rituximab, involving patients with childhood-onset SDNS. Because of the nature of the

intervention, clinical investigators and patients will not be blinded to group assignment. Patients will be recruited from 22 tertiary nephrology care centres in France (Table 1).

Eligibility criteria

Study inclusion criteria comprises the following:

1) Childhood onset nephrotic syndrome (first flare <18 years old)
2) ≥ 2 years old at inclusion
3) Steroid-dependent: patient with at least 2 relapses confirmed during corticosteroids tapering or within 2 weeks following steroids discontinuation, or patient with at least 2 relapses including one under steroid-sparing agent (MMF, calcineurin inhibitors, cyclophosphamide, levamisole) or within 6 months of treatment withdrawal, or with frequent-relapses: 2 or more relapses within 6 months after initial remission or 4 or more relapses within any 12-month period with a relapse within 3 months prior to inclusion
4) In remission: protein-to-creatinine ratio ≤ 0.2 g/g (≤ 0.02 g/mmol)

Study exclusion criteria comprises the following:

1) Patients with SRNS;
2) Patients with genetic nephrotic syndrome;
3) Patients previously treated with rituximab;
4) Patients with no medical insurance;
5) Prior hepatitis B, hepatitis C or HIV infection or any severe and progressive infection;
6) Severe heart failure / severe and uncontrolled cardiac disease;
7) Pregnancy or breastfeeding,
8) Patients with hyperprolinaemia,
9) Known hypersensitivity to one of the study medications,

10) Scheduled and non-postponable injection of live attenuated vaccine

11) Protected adults

12) Patients with neutrophils < $1.5x10^9$/L and/or platelets < $75x10^9$/L

Outcomes

The primary outcome is the occurrence of the first relapse within 24 months following the initiation of treatment. Within this study, relapse shall be defined as a protein to creatinine ratio of 2 g/g of creatinine (0.2 g/mmol) or higher. Secondly, we will monitor time to first relapse from the beginning of treatment, the total number of relapses occurring during the 24 months follow-up period, the cumulative dose of steroid taken during the 24 months follow-up, calculated as cumulative dose of corticosteroid for the enrolment episode plus the cumulative dose of corticosteroid for each relapse and the adverse events during the study period such as infectious complications, treatment tolerance, nausea, neutropenia.

Screening

When investigators observe a recurrence of INS in study candidate patients, they describe this clinical trial to the relevant subjects and obtain their written consent to participate in the trial.After consent is obtained, screening tests are performed to verify eligibility as a subject. If the eligibility of the patient is confirmed after the screening tests, the patient ia randomized. The randomization must be performed within 3 months of the last relapse.

Randomization

After obtaining written consent from all adults or from both parents of children, randomization will be performed using a web-based application and a secured access (CleanWeb®) in a 1:1 ratio to arm A: single infusion of rituximab (375 mg/m²) or arm B: single infusion of rituximab (375 mg/m²) followed by intravenous polyvalent immunoglobulin once a

month for 5 months according to a computer-generated list of randomly permuted blocks. Randomization and concealment will be achieved using a centralized, secure, computer-generated, interactive, web-response system accessible from each study centre. The randomization time is the study time zero (M0). Blinding was not allowed given the nature of the intervention. However, this lack of blinding is partially counter-balanced by the objective nature of the primary outcome measure (biological criteria), and the final analysis will be blinded to allocation of groups.

Procedures

At day 0, all patients will undergo antibiotic prophylaxis with trimethoprim /sulfamethoxazole 800 mg three times per week until B cell reconstitution. All patients will receive a premedication with methylprednisolone and dexchlorpheniramine or hydroxyzine. Patients in both arms will then receive a single injection of rituximab 375 mg/m².

Patients randomized in arm B will receive two doses of IVIg (1g/kg/day) over the course of two consecutive days beginning at M1. From M2 to M5, patients in arm B will receive 0.75 g/kg/day on two consecutive days per month. Doses shall not exceed 100 g. Depending on respective centre practices and patient tolerance, IVIg will be administered in the centre outpatient clinic or conventional hospitalisation units.

Blood sampling (Table 2)

During the clinical trial period, investigators will perform observation, examination, and blood sampling according to a predetermined schedule. On all days of investigational drug administration, blood samples are taken immediately prior to administration.

For all randomized patients, a monthly biological investigation in a local laboratory including IgG, white blood cell and lymphocyte population count and urine analysis including protein-to-creatinine ratio on a sample will be performed during 6 months or until B cell reconstitution, whichever is longer. Additionally, proteinuria will be evaluated once a week using a urinary dipstick until 12 months after rituximab injection and once every two weeks between 12 and 24 months. If the results are positive, a confirmatory urine analysis will be carried out in laboratory. All patients will also be included in a biorepository including samples for DNA extraction and serum banking. The samples will be taken at M0, M9 (if no relapse before M9) and at M24 (or at the time of relapse).

Follow-up visits will be carried out at M3, M6, M9, M12, M18 and M24 with an additional visit in case of relapse as routinely performed in clinical practice. All outcome measures (relapse, time of relapse, number of relapse, amount of corticosteroid taken) and adverse events will be assessed by the investigating physician during the follow up visits.

Prohibited concomitant medications

Patients are instructed to stop all corticosteroid and immunosuppressive treatment (i.e. MMF, levamisole, tacrolimus, CsA and prednisone) within 8 weeks of beginning the trial. In case of corticosteroids treatment, weekly decrease of the dose will be implemented and stopped after 1 month. In case of treatment with steroid-sparing agent, discontinuation will occur after 8 weeks.

Adverse events

Adverse events are, according to the definitions, any unfavourable or unintended event affecting patients on study. In cases of prolongation of hospitalisation, death or significant clinical sequelae, these events are defined as serious adverse events (SAEs), the occurrence of

which the study sponsor (APHP) and the Data Safety Monitoring Board (DSMB) will be informed at short notice. During protocol treatment, all deaths, all SAEs that are life-threatening and any unexpected SAE must be reported to APHP using the SAE web form within 48 h of the initial observation of the event.

Safety aspects of the study are closely assessed by the DSMB, which receives non blinded data. Moreover, the first relapse of INS has been included as SAE to allow monitoring by the DSMB of any major discrepancy between the treatment groups.

Data management

In the RITUXIVIG trial, data are collected at each study visit. Data collection and data entry in the eCRF database are performed by the site investigators with the help of trained local research staff. A data management plan will be written and follow during all the data management and analysis process

Statistical Methods

Sample Size

The number of subjects required to compare relapse rate at 24 months between the two groups (rituximab and IVIg vs. rituximab alone) was estimated. The relapse rate at 24 months in the "rituximab alone" group is assumed to be approximately 60% based on previous reports.[17,18] Assuming a reduction of 30% of relapse rate at 24 months in the rituximab + IVIg group with a power of 80% and a two-sided type I error of 5%, 42 patients per group are required throughout a 12 months' recruitment period. Considering that the number of lost to follow-up will be relatively low in this population (follow up of patients at 2 years is ~95%), size

will be increased to 45 patients per group to provide an initial power of 80% on the intention to treat population.

Statistical Analysis

The Kaplan-Meier method will be used to describe the risk of relapse during the 24 - month follow-up period and comparison between the two groups will be performed using a log-rank test. We will censor participants at the study end date if they are event free or at the time they leave the study. A similar method will be used to study the time to first relapse. Comparison of the number of relapses and the number of adverse events between the groups will be performed using a Chi-square test and the comparison of the cumulative doses of steroid over the study period will be performed using a Mann-Whitney test. All statistical tests will be two-sided using a significance level of 5%.

Monitoring

Monitoring for quality and regulatory compliance will be performed in each centre by the study coordinator from the study coordinating centre. The frequency depends on inclusion rates, questions, and pending issues from earlier audits: once or twice a year.

Confidentiality and data handling

Data will be handled according to the French law. The eCRFs will be hosted by a service provided into a secure electronic system via a web navigator and protected by an individual password for each investigator and clinical research technician. Participant's identifying information will be replaced by a related sequence of characters to ensure confidentiality. The trial database file will be stored for 15 years. The sponsor is the owner of the data.

Patient and Public Involvement

Patients were not involved in the planning and production of this study.

Ethics and dissemination

The study was approved by the Ethics committee (Comité de Protection des Personnes, CPP) of Ouest I on April 24, 2018 and authorised by the French drug regulatory agency (Agence Nationale de Sécurité du Médicament et des Produits de Santé, ANSM– EudraCT n°2017-000826-36)) on May 17, 2018. A manuscript with the results of the primary study and the secondary aims will be published in a peer-reviewed journal.

Discussion

Childhood onset steroid-dependent or frequently relapsing INS is a chronic disease with a long-lasting course and significant impact on patients' quality of life. There are currently no clear guidelines to choose the best treatment for these patients, and the current treatment strategies are all associated with a high rate of relapse. Therefore, clinical trials testing new strategies of treatment and assessing their long-term effects are needed.

The main goal of the RITUXIVIG trial is to demonstrate the superiority of the association of rituximab and IVIg compared to rituximab alone. This trial has several strengths including its multicentre design, the intravenous administration of the drugs that alleviates concerns about compliance and the choice of a long-term outcome (relapse-free survival at 2 years) compared to previous trials. Despite the trial being open-label, the risk of bias should be low given the absence of non-compliance risk and the objective nature of the primary outcome.

This trial will provide the first assessment of the use of IVIg in patients with INS and inform clinicians on whether IVIg immunomodulatory properties can alter the course of the disease. Finally, this strategy may reduce the risk of infection associated with current strategies by reducing the amount of immunosuppressive drugs used and by the direct protective effect of IVIg against infections.

List of Abbreviations

IVIg: intravenous immunoglobulin, SDNS: steroid-dependent nephrotic syndrome

Declarations

Competing interests

The authors have no conflict of interest related to the study to declare.

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Author contributions

JHo and GD were involved in conception and trial design. JHo, GD and AP were involved in drafting of the article. All others are participating in patient recruitment and follow-up and were involved in critical revision of the article for important intellectual content. All the authors were involved in final approval of the article. Preparing study design, collection, management, analysis and interpretation of data; writing of the report; and the decision to submit the report for publication is the responsibility of APHP, the study sponsor.

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Table 1: List of the participating centres in France

City	Hospital Name
Amiens	CHU d'Amiens
Besançon	CHU de Besançon
Bordeaux	CHU de Bordeaux
Caen	CHU de Caen
Clermont	CHU Clermont Ferrand
Créteil	CHU Henri Mondor
Lille	CHU Jeanne de Flandre
Lyon	Hôpital Mère Enfant
Montpellier	CHU de Montpellier
Nancy	CHU de Nancy
Nantes	CHU de Nantes
Nice	CHU Lenval
Paris	CHU Armand Trousseau
Paris	CHU Tenon
Paris	CHU Necker
Paris	CHU Robert Debré
Reims	CHU de Reims
Rouen	CHU de Rouen
Toulouse	CHU de Toulouse
Tours	CHU de Tours
Lyon	Hôpital Edouard Herriot
Limoges	Hôpital de la mère et de l'enfant
Total	**22**

Table 2. Study timeline

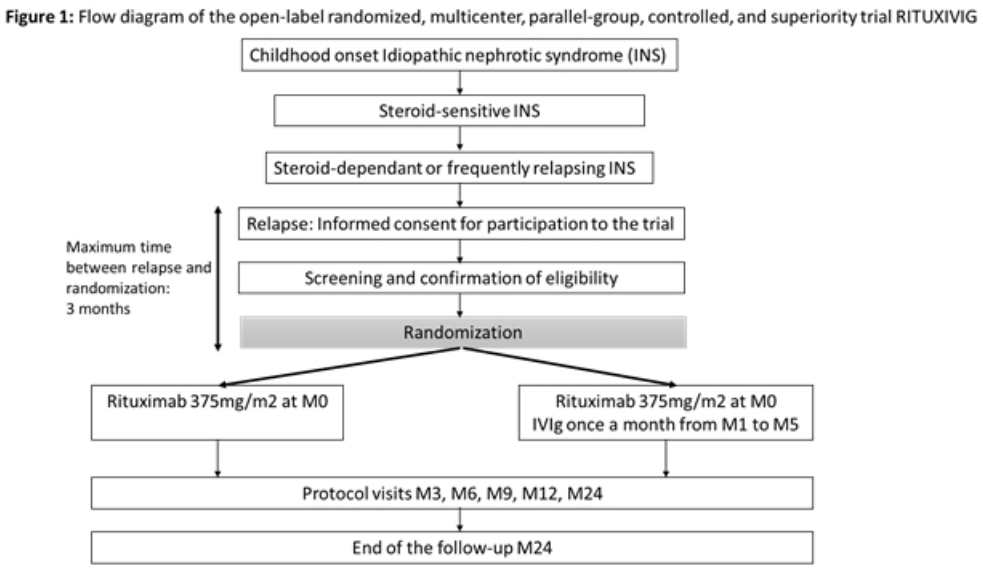
Exams	M0 Inclusion/ randomization	Month 1	2	3	4	5	6	9	12	18	24
Informed consent	○										
History	○										
Clinical exam	○										
Blood Sample for biobanking	○							○ ***			○ ***
Serology (HIV, HBV, HCV)	○										
Haematological exam (total blood count, lymphocyte population count)	○	○	○	○	○	○	○	○	○	○	○
Creatinemia	○	○	○	○	○	○	○	○	○	○	○
SGOT/SGPT, GGT	○										
Serum electrolytes	○	○	○	○	○	○	○	○	○	○	○
Protidemia	○	○	○	○	○	○	○	○	○	○	○
BUN	○	○	○	○	○	○	○	○	○	○	○
Albuminemia	○	○	○	○	○	○	○	○	○	○	○
Proteinuria*	○	○	○	○	○	○	○	○	○	○	○
Creatininuria	○	○	○	○	○	○	○	○	○	○	○
IgG serum level	○	○	○	○	○	○	○	○	○	○	○
Randomization	○										
Rituximab infusion	○										
Hospitalization for IV Immunoglobulin**		○	○	○	○	○					
Follow up visit (consultation)				○			○	○	○	○	○
Relapse		○	○	○	○	○	○	○	○	○	○
Time to first relapse		○	○	○	○	○	○	○	○	○	○
Adverse event	○	○	○	○	○	○	○	○	○	○	○
Pregnancy test****	○										

*Proteinuria is evaluated once a week using a urinary stick until 12 months after rituximab injection and once every two weeks between 12 and 24 months

**If patient randomized in arm B

*** If relapse before M9 biobanking at relapse, if relapse after M9 biobanking at M9 and at relapse

**** for patients at childbearing age



Flow diagram of the open-label randomized, multicenter, parallel-group, controlled, and superiority trial RITUXIVIG

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	X
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	X
	2b	All items from the World Health Organization Trial Registration Data Set	X
Protocol version	3	Date and version identifier	
Funding	4	Sources and types of financial, material, and other support	X
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	X
	5b	Name and contact information for the trial sponsor	X
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	X
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	X
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	X
	6b	Explanation for choice of comparators	X
Objectives	7	Specific objectives or hypotheses	X
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	X

Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	X
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	X
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	X
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	X
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	X
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	X
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	X
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	X
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	X
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	X
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	X

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	X
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	X
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	X
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	X
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	X
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	X
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	X
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	X
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	X
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	X

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	X
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	X
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	X
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	X
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	X
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	X
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	X
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	X
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	X
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	X
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	X
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	X
	31b	Authorship eligibility guidelines and any intended use of professional writers	
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	X

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Efficacy and safety of intravenous immunoglobulin with rituximab versus rituximab alone in childhood-onset steroid-dependent and frequently-relapsing nephrotic syndrome: protocol for a multicentre randomized controlled trial

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Primary Subject Heading:	Renal medicine
Secondary Subject Heading:	Paediatrics
Keywords:	Paediatric nephrology < NEPHROLOGY, Glomerulonephritis < NEPHROLOGY, NEPHROLOGY

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Title: Efficacy and safety of intravenous immunoglobulin with rituximab versus rituximab alone in childhood-onset steroid-dependent and frequently-relapsing nephrotic syndrome: protocol for a multicentre randomized controlled trial

Running title: RITUXIVIG trial

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Abstract

Introduction: Guidelines for the treatment of steroid-dependent nephrotic syndrome (SDNS) and frequently-relapsing nephrotic syndrome (FRNS) are lacking. Given the substantial impact of SDNS/FRNS on quality of life, strategies aiming to provide long-term remission while minimizing treatment side-effects are needed. Several studies confirm that rituximab is effective in preventing early relapses in SDNS/FRNS, however the long-term relapse rate remains high (~70% at 2 years). This trial will assess the association of intravenous immunoglobulins (IVIg) to rituximab in patients with SDNS/FRNS and inform clinicians on whether IVIg immunomodulatory properties can alter the course of the disease and reduce the use of immunosuppressive drugs and their side effects.

Methods and Analysis: We conduct an open-label multicentre, randomized, parallel-group in a 1:1 ratio, controlled, superiority trial to assess the safety and efficacy of a single infusion of rituximab followed by IVIg compared to rituximab alone in childhood-onset FRNS/SDNS. The primary outcome is the occurrence of first relapse within 24 months. Patients are allocated to receive either rituximab alone (375 mg/m²) or rituximab followed by IVIg, which includes an initial immunoglobulin dose of 2g/kg, followed by 1.5g/kg injections once a month for the following five months (Max dose: 100 g).

Ethics and Dissemination: The study has been approved by the Ethics committee (Comité de Protection des Personnes CPP) of Ouest I and authorised by the French drug regulatory agency (Agence Nationale de Sécurité du Médicament et des Produits de Santé, ANSM). Results of the primary study and the secondary aims will be disseminated through peer-reviewed publications.

Trial Registration Number: This trial is registered at ClinicalTrials.gov (NCT03560011)

Key Words: steroid-dependent nephrotic syndrome, immunoglobulin, rituximab

Article Summary

Strengths and Limitations of This Study

- This study will be conducted as a national multicentre randomized controlled trial providing the first reliable data on the use of IVIg in combination with rituximab in patients with idiopathic nephrotic syndrome.
- The lack of blinding of the patients and the physicians is a limitation to the study design, however the objectivity of the primary outcome reduces the risk of bias.
- Intravenous administration of the intervention addresses concerns of non-compliance.

Introduction

Background

Idiopathic nephrotic syndrome (INS) is the first glomerulopathy in children with an incidence estimated between 2 and 3/100,000 inhabitants and a high prevalence of 1/6250 because of the extensive course of the disease. The response to steroid therapy (steroid-sensitive nephrotic syndrome [SSNS] versus steroid-resistant nephrotic syndrome [SRNS]) is of high prognostic significance. Cohort studies including the French NEPHROVIR study found that around 90% of the patients are steroid sensitive.[1,3] However, 60% will become steroid-dependent or frequent-relapsers with a major risk of morbidity related to the complications of the relapses (mostly infections due to immunoglobulin loss and thrombosis) and to the side effects of the treatments used in those patients. The pathophysiology of INS is still incompletely understood. In 1974, Shaloub brought evidence for an immune origin of the disease.[4] Since then, standard immunosuppressive drugs such as calcineurin inhibitors or mycophenolate mofetil (MMF) demonstrated the ability to maintain remission while on treatment. Unfortunately, their effect is only suspensive with 75% of relapse after cyclosporine A (CsA) withdrawal [5] and over 90% of relapse after MMF withdrawal,[6] although maintenance of remission is needed to maintain normal renal function in the long run. Cyclophosphamide demonstrated a long-lasting effect in children with steroid-dependent nephrotic syndrome (SDNS) with a sustained remission rate of 42% at 2 years but its use is limited by its side effects.[7] However, there is currently no consensus on the treatment of SDNS/FRNS and KDIGO guidelines only list potential steroid-sparing agent without giving indication which to prefer.. Several strategies using low dose steroid therapy (once every other day) and the immunosuppressive drugs mentioned previously have been proposed.[8,9] However, they are associated with significant side effects such as diabetes, high blood pressure, infections and renal fibrosis. Moreover, the long duration of the

disease (median time 10 years) has been recently shown to significantly impact the quality of life of patients.[10] Thus treatment and strategies aiming to provide long-term remission while minimizing treatment side-effects in patients with FRNS/SDNS need to be investigated.

In 2004, rituximab (RTX), a humanized anti-CD20 antibody depleting B cells has been reported to induce sustained remission of the nephrotic syndrome in a patient treated for idiopathic thrombocytopenic purpura.[11] Since then, many reports confirmed that RTX is able to induce long-lasting remission even after B cell recovery in patients with SDNS.[12-14] This finding deeply modified our view on the pathophysiology of the disease with the involvement of B cells and not only T cells as previously described. This implication of B cells is further supported by the strong correlation between B cell recovery and INS relapse in patients relapsing after RTX therapy with a recent report underlying the role of memory B cells (CD19+/CD27+).[15]

Two recent randomized trials demonstrated an improvement of the relapse-free survival with RTX when compared with placebo or long-term steroid therapy.[16,17] Similar results have been found in a recently published French randomized controlled trial NEPHRUTIX since the relapse rates at 6 months was 10% in the rituximab arm compared to 100% in the placebo arm.[18] However, the remission rates after two years in patients treated with RTX is only 30 to 40%. Strategies using repeated RTX injection with long B cell depletion duration greatly increase the relapse-free survival rate to over 60% but increase the risk of infection and persistent hypogammaglobulinemia.[13, 19, 20]

Intravenous immunoglobulin (IVIg), which is used for therapeutic purposes, is a polyspecific immunoglobulin IgG preparation purified from plasma pools of several thousand healthy donors. IVIg preparations primarily contain human IgG molecules, with small amounts

of IgA and IgM. The distribution of IgG subclasses in IVIg is comparable to that of IgG in normal serum and the half-life of infused IVIg is approximately three weeks. IVIg was initially used as a substitution for immunoglobulins that were lacking in patients with primary and secondary immune deficiencies. However, since the demonstration in 1981 that IVIg ameliorates immune thrombocytopenic purpura,[21] IVIg is increasingly being used for the treatment of a wide range of autoimmune and systemic inflammatory diseases.[22] In addition to antibody-mediated diseases, IVIg is also effective in several disorders caused by dysregulation of cellular immunity, such as Kawasaki disease, dermatomyositis, multiple sclerosis, graft versus host disease in recipients of allogeneic bone marrow transplants.[23] Clinically, the beneficial effects of IVIg extend beyond the half-life of infused IgG, therefore, its effects cannot be a result of a passive clearance or competition with pathogenic autoantibodies. Together, these observations evoke the possibility that IVIg therapy induces lasting changes in the cellular compartment of the immune system. Several studies demonstrated the ability of IVIg to modulate B cells immune response in vitro and in vivo through several mechanisms such as apoptosis promotion by modulating BCR signalling after binding to CD22,[24] silencing program induction of B cells and neutralization of cytokines such as the B-cell survival factor (BAFF) and A proliferation inducing ligand (APRIL).[25] In vivo, IVIg therapy in women with recurrent spontaneous abortion is accompanied by a small decrease in the peripheral blood B-cell numbers.[26] Aside from their effects on B cells, IVIg have been found to modulate T cell function especially by expanding and enhancing the functions of regulatory T cells (Treg)[27, 28] and by decreasing T cell activation and proliferation through multiple pathways including Il-2 production inhibition.[29-31] Tha-In et al. found that IVIg were as effective as calcineurin inhibitors to

inhibit T cells proliferation in vitro and also impact dendritic cells functions.[32] Many studies also report effects of IVIg on innate immune system.[33]

Thus, we hypothesised that the adjunction of IVIg to a single course of rituximab may further modulate B-cells function and allow a prolonged effect on INS without the need for long-lasting B-cell depletion. Moreover, maintaining a high IgG level may be beneficial in decreasing the risk of infection in those vulnerable patients. Treatment modality was derived from the protocol commonly used to treat antibody-mediated rejection in renal transplant recipients both in adults and in children.[34] In a retrospective pilot study comparing 12 patients treated with RTX and IVIg to 32 controls receiving one injection of RTX alone, we found a great improvement of relapse-free survival at two years from 40% in the RTX alone group to 70% in group receiving both RTX and IVIg with the difference remaining significant after adjustment for age, associated immunosuppressive treatments and B cell depletion duration [unpublished observations, J Hogan]. The proposed clinical trial aims to establish evidence for the use of IVIg in addition to rituximab in patients with frequently relapsing and steroid-dependant nephrotic syndrome.

Methods/Design

Objectives

Primary objective

Our primary objective is to assess the effect of a single infusion of rituximab followed by immunoglobulin injections (once a month during 5 months) on the occurrence of the first relapse within 24 months following the initiation of treatment in patient with childhood onset FRNS/SDNS compared to a single infusion of rituximab.

Secondary objectives

To compare the time to first relapse

To compare the total number of relapse over the 24 months of follow-up

To compare the cumulative dose of steroid over the 24 months of follow-up

To compare the tolerance and safety of the two strategies

Our hypothesis is that the adjunction of intravenous immunoglobulin to rituximab to treat patients with FRNS/SDNS will induce sustained remission of proteinuria even after oral treatment withdrawal and will improve relapse-free survival when compared to rituximab used alone.

Study design (Figure 1)

The trial will be an open-label multicentre, randomized, parallel-group in a 1:1 ratio, controlled, and superiority trial testing a single infusion of rituximab followed by immunoglobulin injections (once a month during 5 months) compared to a single infusion of rituximab, involving patients with childhood-onset FRNS/SDNS. Because of the nature of the intervention, clinical investigators and patients will not be blinded to group assignment. Patients will be recruited from 22 tertiary nephrology care centres in France (Table 1). Inclusions started in April 2019 and are expected to be completed in April 2021. The expected stud completion date is April 2023.

Eligibility criteria

Study inclusion criteria comprises the following:

1) Childhood onset nephrotic syndrome (first flare <18 years old)
2) ≥ 2 years old at inclusion
3) Steroid-dependent: patient with at least 2 relapses confirmed during corticosteroids tapering or within 2 weeks following steroids discontinuation, or patient with at least 2

relapses including one under steroid-sparing agent (MMF, calcineurin inhibitors, cyclophosphamide, levamisole) or within 6 months of treatment withdrawal

4) Frequent-relapsers: 2 or more relapses within 6 months after initial remission or 4 or more relapses within any 12-month period with a relapse within 3 months prior to inclusion

5) In remission: protein-to-creatinine ratio ≤ 0.2 g/g (≤ 0.02 g/mmol)

Study exclusion criteria comprises the following:

1) Patients with SRNS;
2) Patients with genetic mutations known to be associated with nephrotic syndrome;
3) Presence of another active glomerular disease
4) Patients previously treated with rituximab;
5) Patients with no medical insurance;
6) Prior hepatitis B, hepatitis C or HIV infection or any severe and progressive infection;
7) Known Congestive heart failure, left ventricular hypertrophy, or cardiomyopathy;
8) Pregnancy or breastfeeding (a pregnancy test is perform before inclusion in the study in women of childbearing age and effective contraception will be given to these patients at inclusion. This contraception will be continued for one year after the last infusion of Rituximab),
9) Patients with hyperprolinaemia,
10) Known hypersensitivity to one of the study medications,
11) Scheduled and non-postponable injection of live attenuated vaccine
12) Adults under guardianship
13) Patients with neutrophils < $1.5x10^9$/L and/or platelets < $75x10^9$/L

Outcomes

The primary outcome is the occurrence of the first relapse within 24 months following the initiation of treatment. Within this study, relapse shall be defined as a protein to creatinine ratio of 2 g/g of creatinine (0.2 g/mmol) or higher. Secondly, we will monitor time to first relapse from the beginning of treatment, the total number of relapses occurring during the 24 months follow-up period, the cumulative dose of steroid taken during the 24 months follow-up, calculated as cumulative dose of corticosteroid for the enrolment episode plus the cumulative dose of corticosteroid for each relapse, the initiation of a new immunosuppressive therapy and the adverse events during the study period such as infectious complications, treatment tolerance, nausea, neutropenia.

Screening

When investigators observe a recurrence of INS in study candidate patients, they describe this clinical trial to the relevant subjects and obtain their written consent to participate in the trial. After consent is obtained, screening tests are performed to verify eligibility as a subject. If the eligibility of the patient is confirmed after the screening tests, the patient ia randomized. The randomization must be performed within 3 months of the last relapse.

Randomization

After obtaining written consent from all adults or from both parents of children, randomization will be performed using a web-based application and a secured access (CleanWeb®) in a 1:1 ratio to arm A: single infusion of rituximab (375 mg/m²) or arm B: single infusion of rituximab (375 mg/m²) followed by intravenous polyvalent immunoglobulin once a month for 5 months according to a computer-generated list of randomly permuted blocks (mixed blocks). No stratification of the randomization was planned. Randomization and concealment

will be achieved using a centralized, secure, computer-generated, interactive, web-response system accessible from each study centre. The randomization time is the study time zero (M0). Blinding was not allowed given the nature of the intervention. However, this lack of blinding is partially counter-balanced by the objective nature of the primary outcome measure (biological criteria), and the final analysis will be blinded to allocation of groups.

Procedures

At day 0, all patients will undergo antibiotic prophylaxis with trimethoprim /sulfamethoxazole 800 mg three times per week until B cell reconstitution. All patients will receive a premedication with methylprednisolone and dexchlorpheniramine or hydroxyzine. Patients in both arms will then receive a single injection of rituximab 375 mg/m².

Patients randomized in arm B will receive two doses of IVIg (1g/kg/day) over the course of two consecutive days beginning at M1. From M2 to M5, patients in arm B will receive 0.75 g/kg/day on two consecutive days per month. Doses shall not exceed 100 g. Depending on respective centre practices and patient tolerance, IVIg will be administered in the centre outpatient clinic or conventional hospitalisation units.

Blood sampling (Table 2)

During the clinical trial period, investigators will perform observation, examination, and blood sampling according to a predetermined schedule. On all days of investigational drug administration, blood samples are taken immediately prior to administration.

For all randomized patients, a monthly biological investigation in a local laboratory including IgG, white blood cell and lymphocyte population count and urine analysis including protein-to-creatinine ratio on a sample will be performed during 6 months or until B cell

reconstitution, whichever is longer. Additionally, proteinuria will be evaluated once a week using a first-AM urinary dipstick until 12 months after rituximab injection and once every two weeks between 12 and 24 months. If the results are positive, a confirmatory urine analysis will be carried out in laboratory. All patients will also be included in a biorepository including samples for DNA extraction and serum banking. The samples will be taken at M0, M9 (if no relapse before M9) and at M24 (or at the time of relapse).

Follow-up visits will be carried out at M3, M6, M9, M12, M18 and M24 with an additional visit in case of relapse as routinely performed in clinical practice. All outcome measures (relapse, time of relapse, number of relapse, amount of corticosteroid taken) and adverse events will be assessed by the investigating physician during the follow up visits.

Prohibited concomitant medications

Patients are instructed to stop all corticosteroid and immunosuppressive treatment (i.e. MMF, levamisole, tacrolimus, CsA and prednisone) within 8 weeks of beginning the trial. In case of corticosteroids treatment, weekly decrease of the dose will be implemented and stopped after 1 month. In case of treatment with steroid-sparing agent, discontinuation will occur after 8 weeks.

Adverse events

Adverse events are, according to the definitions, any unfavourable or unintended event affecting patients on study. In cases of prolongation of hospitalisation, death or significant clinical sequelae, these events are defined as serious adverse events (SAEs), the occurrence of which the study sponsor (APHP) and the Data Safety Monitoring Board (DSMB) will be informed at short notice. During protocol treatment, all deaths, all SAEs that are life-threatening

and any unexpected SAE must be reported to APHP using the SAE web form within 48 h of the initial observation of the event.

Safety aspects of the study are closely assessed by the DSMB, which receives non blinded data. Moreover, the first relapse of INS has been included as SAE to allow monitoring by the DSMB of any major discrepancy between the treatment groups.

Other adverse events monitored during the follow-up include infections requiring hospitalization, infections not requiring hospitalization, Progressive multifocal leukoencephalopathy, Neutropenia, Acute kidney injury stage 3: increase in creatinine of > or = 200% or eGFR < or = 35ml/min/1.73 m2 (if age < 18 yr) if patients with previously normal renal function, allergic reaction ≥ grade 3 and infusion tolerance.

Data management

In the RITUXIVIG trial, data are collected at each study visit. Data collection and data entry in the eCRF database are performed by the site investigators with the help of trained local research staff. A data management plan will be written and follow during all the data management and analysis process

Statistical Methods

Sample Size

The number of subjects required to compare the proportion of patients with at least one relapse within 24 months between the two groups (rituximab and IVIg vs. rituximab alone) was estimated. The proportion of patients with relapse at 24 months in the "rituximab alone" group is assumed to be approximately 60% based on previous reports.[17,18] Assuming a reduction of

30% in the rituximab + IVIg group with a power of 80% and a two-sided type I error of 5%, 42 patients per group are required throughout a 24 months' recruitment period. Considering that the number of lost to follow-up will be relatively low in this population (follow up of patients at 2 years is ~95%), size will be increased to 45 patients per group to provide an initial power of 80% on the intention to treat population.

Statistical Analysis

The proportion of patients with at least one relapse within 24 months (primary outcome) in the control group and the study group will be compared using a chi-square test. The Kaplan-Meier method will be used to study the time to first relapse and a log-rank test will be used to compare the time to first relapse between the study groups.

Comparison of the number of relapses, the number of adverse events and the cumulative doses of steroids over the study period will be performed using either a log-transform t-test or a Mann-Whitney test based on the distribution of the data. All statistical tests will be two-sided using a significance level of 5%.

Monitoring

Monitoring for quality and regulatory compliance will be performed in each centre by the study coordinator from the study coordinating centre. The frequency depends on inclusion rates, questions, and pending issues from earlier audits: once or twice a year. In addition, quality control of the data is planned to detect missing and inconsistent data. All missing data will be sought in the patients' medical records. If missing data cannot be recovered by the study monitors, a multiple imputation procedure based on a "missing at random" assumption will be considered.

Confidentiality and data handling

Data will be handled according to the French law. The eCRFs will be hosted by a service provided into a secure electronic system via a web navigator and protected by an individual password for each investigator and clinical research technician. Participant's identifying information will be replaced by a related sequence of characters to ensure confidentiality. The trial database file will be stored for 15 years. The sponsor is the owner of the data.

Patient and Public Involvement

Patients were not involved in the planning and production of this study.

Ethics and dissemination

The study was approved by the Ethics committee (Comité de Protection des Personnes, CPP) of Ouest I on April 24, 2018 and authorised by the French drug regulatory agency (Agence Nationale de Sécurité du Médicament et des Produits de Santé, ANSM– EudraCT n°2017-000826-36)) on May 17, 2018. A manuscript with the results of the primary study and the secondary aims will be published in a peer-reviewed journal.

Discussion

Childhood onset steroid-dependent or frequently relapsing INS is a chronic disease with a long-lasting course and significant impact on patients' quality of life. There are currently no clear guidelines to choose the best treatment for these patients, and the current treatment strategies are all associated with a high rate of relapse. Therefore, clinical trials testing new strategies of treatment and assessing their long-term effects are needed.

The main goal of the RITUXIVIG trial is to demonstrate the superiority of the association of rituximab and IVIg compared to rituximab alone. This trial has several strengths including its multicentre design, the intravenous administration of the drugs that alleviates concerns about compliance and the choice of a long-term outcome (relapse-free survival at 2 years) compared to

previous trials. Despite the trial being open-label, the risk of bias should be low given the absence of non-compliance risk and the objective nature of the primary outcome.

This trial will provide the first assessment of the use of IVIg in patients with INS and inform clinicians on whether IVIg immunomodulatory properties can alter the course of the disease. Finally, this strategy may reduce the risk of infection associated with current strategies by reducing the amount of immunosuppressive drugs used and by the direct protective effect of IVIg against infections.

List of Abbreviations

IVIg: intravenous immunoglobulin, SDNS: steroid-dependent nephrotic syndrome, FRNS: Frequently-relapsing nephrotic syndrome.

Declarations

Competing interests

The authors have no conflict of interest related to the study to declare.

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Author contributions

JHo and GD were involved in conception and trial design. JHo, GD and AP were involved in drafting of the article. JHo, ALS, IV, FB, FN, JH, LB, VA, CF, DM, CP, ST, DD, PE, AL, GR, TU, OB, EP, SC, AJ, VG, VB and CD participating in patient recruitment and follow-up and were involved in critical revision of the article for important intellectual content. All the authors

were involved in final approval of the article. Preparing study design, collection, management, analysis and interpretation of data; writing of the report; and the decision to submit the report for publication is the responsibility of APHP, the study sponsor.

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Table 1: List of the participating centres in France

City	Hospital Name
Amiens	CHU d'Amiens
Besançon	CHU de Besançon
Bordeaux	CHU de Bordeaux
Caen	CHU de Caen
Clermont	CHU Clermont Ferrand
Créteil	CHU Henri Mondor
Lille	CHU Jeanne de Flandre
Lyon	Hôpital Mère Enfant
Montpellier	CHU de Montpellier
Nancy	CHU de Nancy
Nantes	CHU de Nantes
Nice	CHU Lenval
Paris	CHU Armand Trousseau
Paris	CHU Tenon
Paris	CHU Necker
Paris	CHU Robert Debré
Reims	CHU de Reims
Rouen	CHU de Rouen
Toulouse	CHU de Toulouse
Tours	CHU de Tours
Lyon	Hôpital Edouard Herriot
Limoges	Hôpital de la mère et de l'enfant
Total	**22**

Table 2. Study timeline

Exams	M0 Inclusion/ randomization	Month 1	2	3	4	5	6	9	12	18	24
Informed consent	○										
History	○										
Clinical exam	○										
Blood Sample for biobanking	○							○ ***			○ ***
Serology (HIV, HBV, HCV)	○										
Haematological exam (total blood count, lymphocyte population count)	○	○	○	○	○	○	○	○	○	○	○
Creatinemia	○	○	○	○	○	○	○	○	○	○	○
SGOT/SGPT, GGT	○										
Serum electrolytes	○	○	○	○	○	○	○	○	○	○	○
Protidemia	○	○	○	○	○	○	○	○	○	○	○
BUN	○	○	○	○	○	○	○	○	○	○	○
Albuminemia	○	○	○	○	○	○	○	○	○	○	○
Proteinuria*	○	○	○	○	○	○	○	○	○	○	○
Creatininuria	○	○	○	○	○	○	○	○	○	○	○
IgG serum level	○	○	○	○	○	○	○	○	○	○	○
Randomization	○										
Rituximab infusion	○										
Hospitalization for IV Immunoglobulin**		○	○	○	○	○					
Follow up visit (consultation)				○			○	○	○	○	○
Relapse		○	○	○	○	○	○	○	○	○	○
Time to first relapse		○	○	○	○	○	○	○	○	○	○
Adverse event	○	○	○	○	○	○	○	○	○	○	○
Pregnancy test****	○										

*Proteinuria is evaluated once a week using a urinary stick until 12 months after rituximab injection and once every two weeks between 12 and 24 months

**If patient randomized in arm B

*** If relapse before M9 biobanking at relapse, if relapse after M9 biobanking at M9 and at relapse

**** for patients at childbearing age

Figures:

Figure 1: Flow diagram of the open-label randomized, multicentre, parallel-group, controlled, and superiority trial RITUXIVIG

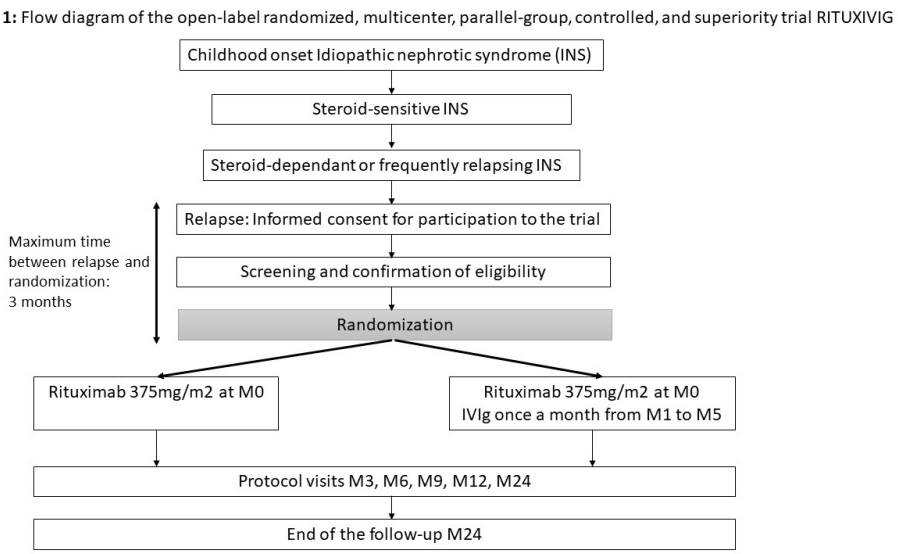

Figure 1: Flow diagram of the open-label randomized, multicentre, parallel-group, controlled, and superiority trial RITUXIVIG

338x190mm (96 x 96 DPI)

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	P1;L1-3
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	P3;L22
	2b	All items from the World Health Organization Trial Registration Data Set	See clinic
Protocol version	3	Date and version identifier	P17;L14-
Funding	4	Sources and types of financial, material, and other support	P17;L16-
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	P18;L1-6
	5b	Name and contact information for the trial sponsor	P18;L8-9
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	P13;L18-P14;L5
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	P5-P8;L2
	6b	Explanation for choice of comparators	P8;L3-P8;L15
Objectives	7	Specific objectives or hypotheses	P8;L18-P9:L8

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	P9;L9-P9;L14
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	P9;L14-P9;L15 + Table1
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	P9;L18-P10;L23
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	P12;L7-P12;L16
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	P13;L11-L16
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	P11;L1-L10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	P9;L14-L17
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	P14;L19-P15;L5
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	
Methods: Assignment of interventions (for controlled trials)			
Allocation:			

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	P11;L17-P12:L2
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	P11;L23-P12;L2
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	P11;L12-L16
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	P12;L3-L5
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	P14;L12-L16
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	P13;L7-L10
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	P15;L15-L22
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	P15;L6-L14
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	P15;L6-L14
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	P15;L18-L22

Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	P13;L18-L5
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	P13;L17-P14;L10
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	P15;L15-L22
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	P16;L8-L12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	P16;L12-L13
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	P11;L12-L13
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	P13;L4-L6
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	P16;L1-L5
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	P17;L13
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	P18;L4-L6
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	P16;L12-L13
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	P18;L10-L12
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	X
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	P12;L17-P13;L6

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Efficacy and safety of intravenous immunoglobulin with rituximab versus rituximab alone in childhood-onset steroid-dependent and frequently-relapsing nephrotic syndrome: protocol for a multicentre randomized controlled trial

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Title: Efficacy and safety of intravenous immunoglobulin with rituximab versus rituximab alone in childhood-onset steroid-dependent and frequently-relapsing nephrotic syndrome: protocol for a multicentre randomized controlled trial

Running title: RITUXIVIG trial

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Abstract

Introduction: Guidelines for the treatment of steroid-dependent nephrotic syndrome (SDNS) and frequently-relapsing nephrotic syndrome (FRNS) are lacking. Given the substantial impact of SDNS/FRNS on quality of life, strategies aiming to provide long-term remission while minimizing treatment side-effects are needed. Several studies confirm that rituximab is effective in preventing early relapses in SDNS/FRNS, however the long-term relapse rate remains high (~70% at 2 years). This trial will assess the association of intravenous immunoglobulins (IVIg) to rituximab in patients with SDNS/FRNS and inform clinicians on whether IVIg immunomodulatory properties can alter the course of the disease and reduce the use of immunosuppressive drugs and their side effects.

Methods and Analysis: We conduct an open-label multicentre, randomized, parallel-group in a 1:1 ratio, controlled, superiority trial to assess the safety and efficacy of a single infusion of rituximab followed by IVIg compared to rituximab alone in childhood-onset FRNS/SDNS. The primary outcome is the occurrence of first relapse within 24 months. Patients are allocated to receive either rituximab alone (375 mg/m²) or rituximab followed by IVIg, which includes an initial immunoglobulin dose of 2g/kg, followed by 1.5g/kg injections once a month for the following five months (Max dose: 100 g).

Ethics and Dissemination: The study has been approved by the Ethics committee (Comité de Protection des Personnes CPP) of Ouest I and authorised by the French drug regulatory agency (Agence Nationale de Sécurité du Médicament et des Produits de Santé, ANSM). Results of the primary study and the secondary aims will be disseminated through peer-reviewed publications.

Trial Registration Number: This trial is registered at ClinicalTrials.gov (NCT03560011)

Key Words: steroid-dependent nephrotic syndrome, frequently-relapsing nephrotic syndrome, immunoglobulin, rituximab

Article Summary

Strengths and Limitations of This Study

- This study will be conducted as a national multicentre randomized controlled trial providing the first reliable data on the use of IVIg in combination with rituximab in patients with idiopathic nephrotic syndrome.
- The lack of blinding of the patients and the physicians is a limitation to the study design, however the objectivity of the primary outcome reduces the risk of bias.
- Intravenous administration of the intervention addresses concerns of non-compliance.

Introduction

Background

Idiopathic nephrotic syndrome (INS) is the first glomerulopathy in children with an incidence estimated between 2 and 3/100,000 inhabitants and a high prevalence of 1/6250 because of the extensive course of the disease. The response to steroid therapy (steroid-sensitive nephrotic syndrome [SSNS] versus steroid-resistant nephrotic syndrome [SRNS]) is of high prognostic significance. Cohort studies including the French NEPHROVIR study found that around 90% of the patients are steroid sensitive.[1,2] However, 60% will become steroid-dependent or frequent-relapsers with a major risk of morbidity related to the complications of the relapses (mostly infections due to immunoglobulin loss and thrombosis) and to the side effects of the treatments used in those patients. The pathophysiology of INS is still incompletely understood. In 1974, Shaloub brought evidence for an immune origin of the disease.[3] Since then, standard immunosuppressive drugs such as calcineurin inhibitors or mycophenolate mofetil (MMF) demonstrated the ability to maintain remission while on treatment. Unfortunately, their effect is only suspensive with 75% of relapse after cyclosporine A (CsA) withdrawal [4] and over 90% of relapse after MMF withdrawal,[5] although maintenance of remission is needed to maintain normal renal function in the long run. Cyclophosphamide demonstrated a long-lasting effect in children with steroid-dependent nephrotic syndrome (SDNS) with a sustained remission rate of 42% at 2 years but its use is limited by its side effects.[6] However, there is currently no consensus on the treatment of SDNS/FRNS and KDIGO guidelines only list potential steroid-sparing agent without giving indication which to prefer. Several strategies using low dose steroid therapy (once every other day) and the immunosuppressive drugs mentioned previously have been proposed.[7,8] However, they are associated with significant side effects such as diabetes, high blood pressure, infections and renal fibrosis. Moreover, the long duration of the disease

(median time 10 years) has been recently shown to significantly impact the quality of life of patients.[9] Thus treatment and strategies aiming to provide long-term remission while minimizing treatment side-effects in patients with FRNS/SDNS need to be investigated.

In 2004, rituximab (RTX), a humanized anti-CD20 antibody depleting B cells has been reported to induce sustained remission of the nephrotic syndrome in a patient treated for idiopathic thrombocytopenic purpura.[10] Since then, many reports confirmed that RTX is able to induce long-lasting remission even after B cell recovery in patients with SDNS.[11-13] This finding deeply modified our view on the pathophysiology of the disease with the involvement of B cells and not only T cells as previously described. This implication of B cells is further supported by the strong correlation between B cell recovery and INS relapse in patients relapsing after RTX therapy with a recent report underlying the role of memory B cells (CD19+/CD27+).[14]

Two recent randomized trials demonstrated an improvement of the relapse-free survival with RTX when compared with placebo or long-term steroid therapy.[15,16] Similar results have been found in a recently published French randomized controlled trial NEPHRUTIX since the relapse rates at 6 months was 10% in the rituximab arm compared to 100% in the placebo arm.[17] However, the remission rates after two years in patients treated with RTX is only 30 to 40%. Strategies using repeated RTX injection with long B cell depletion duration greatly increase the relapse-free survival rate to over 60% but increase the risk of infection and persistent hypogammaglobulinemia.[12, 18, 19]

Intravenous immunoglobulin (IVIg), which is used for therapeutic purposes, is a polyspecific immunoglobulin IgG preparation purified from plasma pools of several thousand healthy donors. IVIg preparations primarily contain human IgG molecules, with small amounts

of IgA and IgM. The distribution of IgG subclasses in IVIg is comparable to that of IgG in normal serum and the half-life of infused IVIg is approximately three weeks. IVIg was initially used as a substitution for immunoglobulins that were lacking in patients with primary and secondary immune deficiencies. However, since the demonstration in 1981 that IVIg ameliorates immune thrombocytopenic purpura,[20] IVIg is increasingly being used for the treatment of a wide range of autoimmune and systemic inflammatory diseases.[21] In addition to antibody-mediated diseases, IVIg is also effective in several disorders caused by dysregulation of cellular immunity, such as Kawasaki disease, dermatomyositis, multiple sclerosis, graft versus host disease in recipients of allogeneic bone marrow transplants.[22] Clinically, the beneficial effects of IVIg extend beyond the half-life of infused IgG, therefore, its effects cannot be a result of a passive clearance or competition with pathogenic autoantibodies. Together, these observations evoke the possibility that IVIg therapy induces lasting changes in the cellular compartment of the immune system. Several studies demonstrated the ability of IVIg to modulate B cells immune response in vitro and in vivo through several mechanisms such as apoptosis promotion by modulating BCR signalling after binding to CD22,[23] silencing program induction of B cells and neutralization of cytokines such as the B-cell survival factor (BAFF) and A proliferation inducing ligand (APRIL).[24] In vivo, IVIg therapy in women with recurrent spontaneous abortion is accompanied by a small decrease in the peripheral blood B-cell numbers.[25] Aside from their effects on B cells, IVIg have been found to modulate T cell function especially by expanding and enhancing the functions of regulatory T cells (Treg)[26, 27] and by decreasing T cell activation and proliferation through multiple pathways including Il-2 production inhibition.[28-30] Tha-In et al. found that IVIg were as effective as calcineurin inhibitors to

inhibit T cells proliferation in vitro and also impact dendritic cells functions.[31] Many studies also report effects of IVIg on innate immune system.[32]

Thus, we hypothesised that the adjunction of IVIg to a single course of rituximab may further modulate B-cells function and allow a prolonged effect on INS without the need for long-lasting B-cell depletion. Moreover, maintaining a high IgG level may be beneficial in decreasing the risk of infection in those vulnerable patients. Treatment modality was derived from the protocol commonly used to treat antibody-mediated rejection in renal transplant recipients both in adults and in children.[33] In a retrospective pilot study comparing 12 patients treated with RTX and IVIg to 32 controls receiving one injection of RTX alone, we found a great improvement of relapse-free survival at two years from 40% in the RTX alone group to 70% in group receiving both RTX and IVIg with the difference remaining significant after adjustment for age, associated immunosuppressive treatments and B cell depletion duration [unpublished observations, J Hogan]. The proposed clinical trial aims to establish evidence for the use of IVIg in addition to rituximab in patients with frequently relapsing and steroid-dependant nephrotic syndrome.

Methods/Design

Objectives

Primary objective

Our primary objective is to assess the effect of a single infusion of rituximab followed by immunoglobulin injections (once a month during 5 months) on the occurrence of the first relapse within 24 months following the initiation of treatment in patient with childhood onset FRNS/SDNS compared to a single infusion of rituximab.

Secondary objectives

To compare the time to first relapse

To compare the total number of relapse over the 24 months of follow-up

To compare the cumulative dose of steroid over the 24 months of follow-up

To compare the tolerance and safety of the two strategies

Our hypothesis is that the adjunction of intravenous immunoglobulin to rituximab to treat patients with FRNS/SDNS will induce sustained remission of proteinuria even after oral treatment withdrawal and will improve relapse-free survival when compared to rituximab used alone.

Study design

The trial will be an open-label multicentre, randomized, parallel-group in a 1:1 ratio, controlled, and superiority trial testing a single infusion of rituximab followed by immunoglobulin injections (once a month during 5 months) compared to a single infusion of rituximab, involving patients with childhood-onset FRNS/SDNS (Figure 1). Because of the nature of the intervention, clinical investigators and patients will not be blinded to group assignment. Patients will be recruited from 22 tertiary nephrology care centres in France (Table 1). Inclusions started in April 2019 and are expected to be completed in April 2021. The expected stud completion date is April 2023.

Eligibility criteria

Study inclusion criteria comprises the following:

1) Childhood onset nephrotic syndrome (first flare <18 years old)
2) ≥ 2 years old at inclusion
3) Steroid-dependent: patient with at least 2 relapses confirmed during corticosteroids tapering or within 2 weeks following steroids discontinuation, or patient with at least 2

relapses including one under steroid-sparing agent (MMF, calcineurin inhibitors, cyclophosphamide, levamisole) or within 6 months of treatment withdrawal

4) Frequent-relapsers: 2 or more relapses within 6 months after initial remission or 4 or more relapses within any 12-month period with a relapse within 3 months prior to inclusion

5) In remission: protein-to-creatinine ratio ≤ 0.2 g/g (≤ 0.02 g/mmol)

Study exclusion criteria comprises the following:

1) Patients with SRNS;
2) Patients with genetic mutations known to be associated with nephrotic syndrome;
3) Presence of another active glomerular disease
4) Patients previously treated with rituximab;
5) Patients with no medical insurance;
6) Prior hepatitis B, hepatitis C or HIV infection or any severe and progressive infection;
7) Known Congestive heart failure, left ventricular hypertrophy, or cardiomyopathy;
8) Pregnancy or breastfeeding (a pregnancy test is perform before inclusion in the study in women of childbearing age and effective contraception will be given to these patients at inclusion. This contraception will be continued for one year after the last infusion of Rituximab),
9) Patients with hyperprolinaemia,
10) Known hypersensitivity to one of the study medications,
11) Scheduled and non-postponable injection of live attenuated vaccine
12) Adults under guardianship
13) Patients with neutrophils < $1.5x10^9$/L and/or platelets < $75x10^9$/L

Outcomes

The primary outcome is the occurrence of the first relapse within 24 months following the initiation of treatment. Within this study, relapse shall be defined as a protein to creatinine ratio of 2 g/g of creatinine (0.2 g/mmol) or higher. No clinical manifestation is requested to define relapse. Secondly, we will monitor time to first relapse from the beginning of treatment, the total number of relapses occurring during the 24 months follow-up period, the cumulative dose of steroid taken during the 24 months follow-up, calculated as cumulative dose of corticosteroid for the enrolment episode plus the cumulative dose of corticosteroid for each relapse, the initiation of a new immunosuppressive therapy and the adverse events during the study period such as infectious complications, treatment tolerance, nausea, neutropenia.

Screening

When investigators observe a recurrence of INS in study candidate patients, they describe this clinical trial to the relevant subjects and obtain their written consent to participate in the trial. After consent is obtained, screening tests are performed to verify eligibility as a subject. If the eligibility of the patient is confirmed after the screening tests, the patient is randomized. The randomization must be performed within 3 months of the last relapse.

Randomization

After obtaining written consent from all adults or from both parents of children (Supplemental material), randomization will be performed using a web-based application and a secured access (CleanWeb®) in a 1:1 ratio to arm A: single infusion of rituximab (375 mg/m²) or arm B: single infusion of rituximab (375 mg/m²) followed by intravenous polyvalent immunoglobulin once a month for 5 months according to a computer-generated list of randomly permuted blocks (mixed blocks). No stratification of the randomization was planned.

Randomization and concealment will be achieved using a centralized, secure, computer-generated, interactive, web-response system accessible from each study centre. The randomization time is the study time zero (M0). Blinding was not allowed given the nature of the intervention. However, this lack of blinding is partially counter-balanced by the objective nature of the primary outcome measure (biological criteria), and the final analysis will be blinded to allocation of groups.

Procedures

At day 0, all patients will undergo antibiotic prophylaxis with trimethoprim /sulfamethoxazole 800 mg three times per week until B cell reconstitution. All patients will receive a premedication with methylprednisolone and dexchlorpheniramine or hydroxyzine. Patients in both arms will then receive a single injection of rituximab 375 mg/m².

Patients randomized in arm B will receive two doses of IVIg (1g/kg/day) over the course of two consecutive days beginning at M1. From M2 to M5, patients in arm B will receive 0.75 g/kg/day on two consecutive days per month. Doses shall not exceed 100 g. Depending on respective centre practices and patient tolerance, IVIg will be administered in the centre outpatient clinic or conventional hospitalisation units.

Blood sampling (Table 2)

During the clinical trial period, investigators will perform observation, examination, and blood sampling according to a predetermined schedule. On all days of investigational drug administration, blood samples are taken immediately prior to administration.

For all randomized patients, a monthly biological investigation in a local laboratory including IgG, white blood cell and lymphocyte population count and urine analysis including

protein-to-creatinine ratio on a sample will be performed during 6 months or until B cell reconstitution, whichever is longer. Additionally, proteinuria will be evaluated once a week using a first-AM urinary dipstick until 12 months after rituximab injection and once every two weeks between 12 and 24 months. If the results are positive, a confirmatory urine analysis will be carried out in laboratory. All patients will also be included in a biorepository including samples for DNA extraction and serum banking. The samples will be taken at M0, M9 (if no relapse before M9) and at M24 (or at the time of relapse).

Follow-up visits will be carried out at M3, M6, M9, M12, M18 and M24 with an additional visit in case of relapse as routinely performed in clinical practice. All outcome measures (relapse, time of relapse, number of relapse, amount of corticosteroid taken) and adverse events will be assessed by the investigating physician during the follow up visits.

Prohibited concomitant medications

Patients are instructed to stop all corticosteroid and immunosuppressive treatment (i.e. MMF, levamisole, tacrolimus, CsA and prednisone) within 8 weeks of beginning the trial. In case of corticosteroids treatment, weekly decrease of the dose will be implemented and stopped after 1 month. In case of treatment with steroid-sparing agent, discontinuation will occur after 8 weeks.

Adverse events

Adverse events are, according to the definitions, any unfavourable or unintended event affecting patients on study. In cases of prolongation of hospitalisation, death or significant clinical sequelae, these events are defined as serious adverse events (SAEs), the occurrence of which the study sponsor (APHP) and the Data Safety Monitoring Board (DSMB) will be informed at short notice. During protocol treatment, all deaths, all SAEs that are life-threatening

and any unexpected SAE must be reported to APHP using the SAE web form within 48 h of the initial observation of the event.

Safety aspects of the study are closely assessed by the DSMB, which receives non blinded data. Moreover, the first relapse of INS has been included as SAE to allow monitoring by the DSMB of any major discrepancy between the treatment groups.

Other adverse events monitored during the follow-up include infections requiring hospitalization, infections not requiring hospitalization, Progressive multifocal leukoencephalopathy, Neutropenia, Acute kidney injury stage 3: increase in creatinine of > or = 200% or eGFR < or = 35ml/min/1.73 m2 (if age < 18 yr) if patients with previously normal renal function, allergic reaction ≥ grade 3 and infusion tolerance.

Data management

In the RITUXIVIG trial, data are collected at each study visit. Data collection and data entry in the eCRF database are performed by the site investigators with the help of trained local research staff. A data management plan will be written and follow during all the data management and analysis process

Statistical Methods

Sample Size

The number of subjects required to compare the proportion of patients with at least one relapse within 24 months between the two groups (rituximab and IVIg vs. rituximab alone) was estimated. The proportion of patients with relapse at 24 months in the "rituximab alone" group is assumed to be approximately 60% based on previous reports.[17,18] Assuming a reduction of

30% in the rituximab + IVIg group with a power of 80% and a two-sided type I error of 5%, 42 patients per group are required throughout a 24 months' recruitment period. Considering that the number of lost to follow-up will be relatively low in this population (follow up of patients at 2 years is ~95%), size will be increased to 45 patients per group to provide an initial power of 80% on the intention to treat population.

Statistical Analysis

The proportion of patients with at least one relapse within 24 months (primary outcome) in the control group and the study group will be compared using a chi-square test. The Kaplan-Meier method will be used to study the time to first relapse and a log-rank test will be used to compare the time to first relapse between the study groups.

Comparison of the number of relapses, the number of adverse events and the cumulative doses of steroids over the study period will be performed using either a log-transform t-test for normally distributed variables or a Mann-Whitney test for non-normally distributed variables (normality will be tested using a Kolmogorov-Smirnov test). All statistical tests will be two-sided using a significance level of 5%.

Monitoring

Monitoring for quality and regulatory compliance will be performed in each centre by the study coordinator from the study coordinating centre. The frequency depends on inclusion rates, questions, and pending issues from earlier audits: once or twice a year. In addition, quality control of the data is planned to detect missing and inconsistent data. All missing data will be sought in the patients' medical records. If missing data cannot be recovered by the study monitors, a multiple imputation procedure based on a "missing at random" assumption using a fully conditional specification method will be considered.

Confidentiality and data handling

Data will be handled according to the French law. The eCRFs will be hosted by a service provided into a secure electronic system via a web navigator and protected by an individual password for each investigator and clinical research technician. Participant's identifying information will be replaced by a related sequence of characters to ensure confidentiality. The trial database file will be stored for 15 years. The sponsor is the owner of the data.

Patient and Public Involvement

Patients were not involved in the planning and production of this study.

Ethics and dissemination

The study was approved by the Ethics committee (Comité de Protection des Personnes, CPP) of Ouest I on April 24, 2018 and authorised by the French drug regulatory agency (Agence Nationale de Sécurité du Médicament et des Produits de Santé, ANSM– EudraCT n°2017-000826-36)) on May 17, 2018. A manuscript with the results of the primary study and the secondary aims will be published in a peer-reviewed journal.

Discussion

Childhood onset steroid-dependent or frequently relapsing INS is a chronic disease with a long-lasting course and significant impact on patients' quality of life. There are currently no clear guidelines to choose the best treatment for these patients, and the current treatment strategies are all associated with a high rate of relapse. Therefore, clinical trials testing new strategies of treatment and assessing their long-term effects are needed.

The main goal of the RITUXIVIG trial is to demonstrate the superiority of the association of rituximab and IVIg compared to rituximab alone. This trial has several strengths including its multicentre design, the intravenous administration of the drugs that alleviates concerns about

compliance and the choice of a long-term outcome (relapse-free survival at 2 years) compared to previous trials. Despite the trial being open-label, the risk of bias should be low given the absence of non-compliance risk and the objective nature of the primary outcome.

This trial will provide the first assessment of the use of IVIg in patients with INS and inform clinicians on whether IVIg immunomodulatory properties can alter the course of the disease. Finally, this strategy may reduce the risk of infection associated with current strategies by reducing the amount of immunosuppressive drugs used and by the direct protective effect of IVIg against infections.

List of Abbreviations

IVIg: intravenous immunoglobulin, SDNS: steroid-dependent nephrotic syndrome, FRNS: Frequently-relapsing nephrotic syndrome.

Declarations

Competing interests

The authors have no conflict of interest related to the study to declare.

Funding

The RITUXIVIG trial is funded by a grant from Programme Hospitalier de Recherche Clinique - PHRC 2016 (Ministry of Health).

Author contributions

JHo and GD were involved in conception and trial design and SGC was involved in designing the analytical plan. JHo, GD and AP were involved in drafting of the article. JHo, ALS, IV, FB, FN, JH, LB, VA, CF, DM, CP, ST, DD, PE, AL, GR, TU, OB, EP, SC, AJ, VG, VB and CD

participating in patient recruitment and follow-up and were involved in critical revision of the article for important intellectual content. All the authors were involved in final approval of the article. Preparing study design, collection, management, analysis and interpretation of data; writing of the report; and the decision to submit the report for publication is the responsibility of APHP, the study sponsor.

Acknowledgements

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Table 1: List of the participating centres in France

City	Hospital Name
Amiens	CHU d'Amiens
Besançon	CHU de Besançon
Bordeaux	CHU de Bordeaux
Caen	CHU de Caen
Clermont	CHU Clermont Ferrand
Créteil	CHU Henri Mondor
Lille	CHU Jeanne de Flandre
Lyon	Hôpital Mère Enfant
Montpellier	CHU de Montpellier
Nancy	CHU de Nancy
Nantes	CHU de Nantes
Nice	CHU Lenval
Paris	CHU Armand Trousseau
Paris	CHU Tenon
Paris	CHU Necker
Paris	CHU Robert Debré
Reims	CHU de Reims
Rouen	CHU de Rouen
Toulouse	CHU de Toulouse
Tours	CHU de Tours
Lyon	Hôpital Edouard Herriot
Limoges	Hôpital de la mère et de l'enfant
Total	**22**

Table 2. Study timeline

Exams	M0 Inclusion/ randomization	Month 1	2	3	4	5	6	9	12	18	24
Informed consent	○										
History	○										
Clinical exam	○										
Blood Sample for biobanking	○							○ ***			○ ***
Serology (HIV, HBV, HCV)	○										
Haematological exam (total blood count, lymphocyte population count)	○	○	○	○	○	○	○	○	○	○	○
Creatinemia	○	○	○	○	○	○	○	○	○	○	○
SGOT/SGPT, GGT	○										
Serum electrolytes	○	○	○	○	○	○	○	○	○	○	○
Protidemia	○	○	○	○	○	○	○	○	○	○	○
BUN	○	○	○	○	○	○	○	○	○	○	○
Albuminemia	○	○	○	○	○	○	○	○	○	○	○
Proteinuria*	○	○	○	○	○	○	○	○	○	○	○
Creatininuria	○	○	○	○	○	○	○	○	○	○	○
IgG serum level	○	○	○	○	○	○	○	○	○	○	○
Randomization	○										
Rituximab infusion	○										
Hospitalization for IV Immunoglobulin**		○	○	○	○	○					
Follow up visit (consultation)				○			○	○	○	○	○
Relapse		○	○	○	○	○	○	○	○	○	○
Time to first relapse		○	○	○	○	○	○	○	○	○	○
Adverse event	○	○	○	○	○	○	○	○	○	○	○
Pregnancy test****	○										

*Proteinuria is evaluated once a week using a urinary stick until 12 months after rituximab injection and once every two weeks between 12 and 24 months

**If patient randomized in arm B

*** If relapse before M9 biobanking at relapse, if relapse after M9 biobanking at M9 and at relapse

**** for patients at childbearing age

Figures:

Figure 1: Flow diagram of the open-label randomized, multicentre, parallel-group, controlled, and superiority trial RITUXIVIG

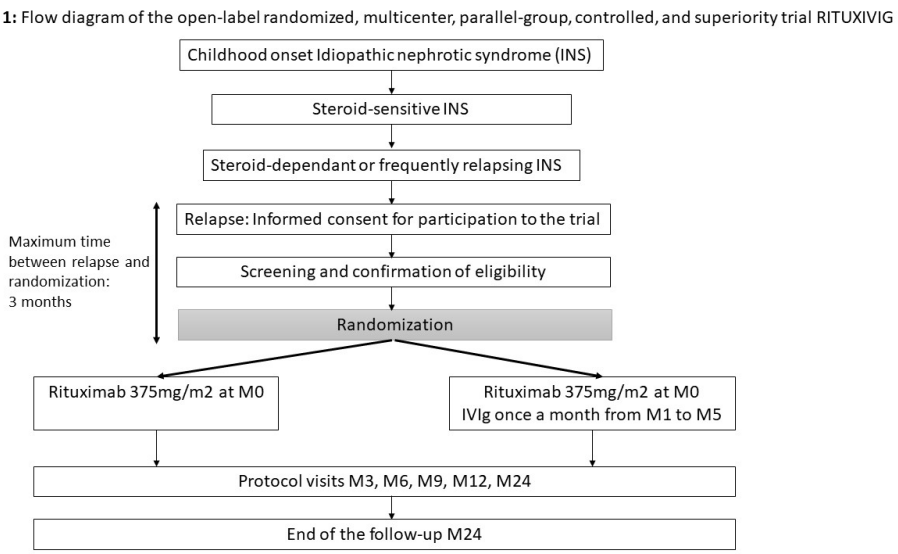

Figure 1: Flow diagram of the open-label randomized, multicentre, parallel-group, controlled, and superiority trial RITUXIVIG

338x190mm (96 x 96 DPI)

ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

Evaluation de l'efficacité et de la tolérance de l'injection d'immunoglobulines en association au rituximab par rapport au rituximab seul dans le syndrome néphrotique corticodépendant à début pédiatrique.

RITUXIVIG

Cette recherche est promue par l'Assistance Publique - Hôpitaux de Paris
Délégation à la Recherche Clinique et à l'Innovation
1 avenue Claude Vellefaux
75010 Paris

NOTE D'INFORMATION (jeunes de 13 à 17 ans)

Le Docteur ……travaillant à l'hôpital ……dans le service de Néphrologie (Tél. ……..), vous propose de participer à une recherche concernant votre maladie.

Lisez bien les explications de cette note d'information. Vous pourrez poser toutes vos questions à votre médecin.

1) Voici pourquoi nous faisons cette recherche et pourquoi nous vous demandons de participer

Vous avez une maladie des reins qui s'appelle syndrome néphrotique corticodépendant. Le traitement de cette maladie par des corticoïdes permet une rémission mais on ne peut pas prolonger ce traitement car il entraîne des effets indésirables. La maladie peut par contre revenir quand on diminue ces corticoïdes ou qu'on les arrête, c'est la rechute.

Il va donc falloir ajouter un traitement supplémentaire pour pouvoir diminuer la dose de corticoïde et si possible l'arrêter. Un des traitements utilisé actuellement est le Rituximab. Un autre traitement, les immunoglobulines, pourrait améliorer l'efficacité du Rituximab. Les médecins souhaitent faire cette étude pour évaluer l'efficacité de l'association du Rituximab et des immunoglobulines

2) Voici comment la recherche va se passer

Cette recherche sera réalisée dans les services de néphrologie de 20 centres hospitaliers en France, chez 90 patients, enfants et adultes. 45 d'entre eux recevront les immunoglobulines ajoutés au rituximab et les 45 autres recevront le rituximab. C'est un tirage au sort qui décidera quel traitement vous recevrez.
Les immunoglobulines tout comme le rituximab sont des anticorps et sont administrés par perfusion intraveineuse.

Au cours de la recherche, vous serez hospitalisé(é) 1 fois si vous recevez le rituximab seul ou 6 fois si vous recevez les immunoglobulines ajoutées au rituximab, soit 1 fois au début pour le rituximab puis 2 jours d'affilés une fois par mois pendant 5 mois pour les perfusions d'immunoglobulines.

La durée de votre participation à cette recherche est de 2 ans. Votre suivi après le(s) traitement(s) sera effectué en consultation avec votre médecin au rythme habituel c'est-à-dire bilan biologiques tous les mois jusqu'au 6ème mois puis tous les 3 mois jusqu'à 1 an puis tous les 6 mois jusqu'à 2 ans.

3) Quels sont les risques ajoutés par la recherche ?

Vous serez hospitalisé(e) durant la prise du(des) traitement(s), le médecin de la recherche fera le nécessaire pour minimiser les éventuels effets secondaires des traitements comme les maux de têtes, les nausées ou les vomissements durant les perfusions. Il y aura également deux prises de sang pour constituer une collection biologique.

4) Quels sont les bénéfices liés à votre participation à cette recherche ?

Le bénéfice attendu de cette recherche est une diminution du risque de rechute lié au syndrome néphrotique. De plus, l'utilisation de médicament comme les immunoglobulines qui sont des anticorps pourrait diminuer le risque d'infection associé à tous les traitements immunosuppresseurs. Le traitement proposé permet également l'arrêt des traitements oraux.

Par ailleurs, la constitution d'une collection biologique avec vos prélèvements sanguins permettra de contribuer à une meilleure connaissance du syndrome néphrotique, des mécanismes de cette maladie et des traitements à proposer aux patients.

5) Vous n'êtes pas obligé(e) de participer à cette recherche.

Si vous décidez de ne pas participer à ce protocole, votre médecin pourra vous proposer pour la prise en charge de votre syndrome néphrotique corticodépendant, soit l'introduction ou la poursuite d'un traitement suspensif comme le Cellcept, le Néoral ou le Prograf associé ou non à la poursuite du traitement par corticoïdes, soit un traitement par rituximab seul.

Si vous décidez de participer à cette recherche, vous pourrez décider d'arrêter par la suite à n'importe quel moment. Votre médecin continuera à bien s'occuper de vous et gardera de très bonnes relations avec vos parents, comme auparavant.

Vous devez savoir également que le médecin qui vous suit peut, lui aussi, décider d'arrêter votre participation à la recherche s'il juge que c'est mieux pour vous. Si cela arrive il vous en expliquera les raisons.

Votre identité (nom et prénom) restera secrète et ne sera pas communiquée à d'autres personnes que l'équipe à l'hôpital.

6) Si vous acceptez de participer à cette recherche, vous pouvez signer le document appelé "consentement" avec vos parents.

N'hésitez pas à interroger votre médecin pour obtenir des réponses aux questions que vous vous posez.

Nom, prénom du mineur :

Date de l'entretien d'information :

Ce document est à réaliser en 2 exemplaires, dont l'original doit être conservé pendant 15 ans par l'investigateur, le deuxième remis au mineur ou à ses parents

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	P1;L1-3
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	P3;L22
	2b	All items from the World Health Organization Trial Registration Data Set	See clinic
Protocol version	3	Date and version identifier	P17;L14-
Funding	4	Sources and types of financial, material, and other support	P17;L16-
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	P18;L1-6
	5b	Name and contact information for the trial sponsor	P18;L8-9
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	P13;L18-P14;L5
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	P5-P8;L2
	6b	Explanation for choice of comparators	P8;L3-P8;L15
Objectives	7	Specific objectives or hypotheses	P8;L18-P9:L8

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	P9;L9-P9;L14
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	P9;L14-P9;L15 + Table1
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	P9;L18-P10;L23
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	P12;L7-P12;L16
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	N/A
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	P13;L11-L16
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	P11;L1-L10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	P9;L14-L17
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	P14;L19-P15;L5
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	
Methods: Assignment of interventions (for controlled trials)			
Allocation:			

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	P11;L17-P12:L2
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	P11;L23-P12;L2
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	P11;L12-L16
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	P12;L3-L5
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	P14;L12-L16
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	P13;L7-L10
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	P15;L15-L22
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	P15;L6-L14
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	P15;L6-L14
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	P15;L18-L22

Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	P13;L18-L5
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	P13;L17-P14;L10
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	P15;L15-L22
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	P16;L8-L12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	P16;L12-L13
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	P11;L12-L13
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	P13;L4-L6
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	P16;L1-L5
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	P17;L13
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	P18;L4-L6
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	P16;L12-L13
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	P18;L10-L12
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	X
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	P12;L17-P13;L6

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.